Biochimica et Biophysica Acta 1842 (2014) 734–738

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Psoriasis pathogenesis — Pso p27 is generated from SCCA1 with chymase

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ARTICLE INFO

Article history:
Received 4 November 2013
Received in revised form 4 February 2014
Accepted 11 February 2014
Available online 18 February 2014

Keywords:
Autoimmunity
Chymase
Pathogenesis
Pso p27
Psoriasis
SCCA1

ABSTRACT

Psoriasis is a chronic inflammatory skin disease with unknown aetiology. Infiltration of inflammatory cells as the initial event in the development of new psoriatic plaques together with the defined inflamed areas of such lesions argues for an immunological disease with a local production of a causal antigen. The auto-antigen Pso p27 is a protein expressed in the skin lesions. We recently demonstrated that Pso p27 is homologous to the core amino acid sequences of squamous cell carcinoma antigens 1 and 2 (SCCA1/2) and it is apparently generated from SCCA molecules by digestion with highly specific endoproteases. In this communication we demonstrate the generation of Pso p27 from SCCA1 with extracts from psoriatic scale and even more remarkably, the generation of Pso p27 from SCCA1 in the presence of mast cell associated chymase. These findings open up for new therapeutic strategies in psoriasis and probably also in other autoimmune diseases as Pso p27 epitopes have been detected in diseased tissues from patients with various chronic inflammatory diseases.

1. Introduction

Psoriasis is a chronic inflammatory skin disease which afflicts about 2% of the general population. The association between psoriasis and selected MHC molecules and the defined skin lesions argues for an immunologic disease caused by a specific and locally expressed antigen [1,2].

Pso p27 is a protein detected in mast cells in psoriatic lesions and extractable from psoriatic scale, while it is not present in uninvolved psoriatic skin or skin from healthy controls [3–5]. The presence of specific antibodies against Pso p27 in psoriatic scale makes Pso p27 a candidate as causal agent in the immune reactions in psoriasis [4]. Sequencing of Pso p27 has demonstrated homologies with squamous cell carcinoma antigens (SCCAs). This includes conformities with SCCA1 (serpin B3) as well as SCCA2 (serpin B4) [6]. Pso p27 is a smaller protein compared to SCCA molecules, and analyses have shown that the N-terminal and the C-terminal ends of the SCCA molecules are not present in Pso p27. Based on this knowledge we suggested that Pso p27 is generated by posttranslational modification of the SCCA molecules [6]. This was substantiated through indirect immunofluorescence analysis of skin biopsies from psoriatic patients using specific antiserum against the N-terminal end of SCCA1 and specific monoclonal antibodies against Pso p27 [7]. These observations give rise to the assumption that Pso p27 is generated from the SCCA molecules in dermal cells and at the surface of the epidermis.

In this communication we demonstrate the generation of Pso p27 from recombinant SCCA1 by specific endoproteolytic activity in extracts from psoriatic scale and with mast cell associated chymase.

2. Materials and methods

2.1. Indirect immune fluorescence of biopsies obtained from psoriatic skin lesions

Skin punch biopsies were taken from psoriatic lesions and snap frozen in liquid nitrogen. Thin sections from the biopsies were cut and stored at −80 °C. Prior to indirect immunofluorescence analysis the sections were air dried at room temperature and fixed for 15 min in acetone. The sections were incubated with rabbit polyclonal anti-SCCA antiserum (ab47726 Abcam), 20 μg/ml and biotinylated mouse monoclonal anti-Pso p27 antibody (3A3D10), 8 μg/ml [8,9], diluted in phosphate buffered saline containing 0.05% Tween 20 (PBS-T). After washing with PBS-T the sections were incubated with fluorescein isothiocyanate conjugated swine anti-rabbit antibodies (DAKO) and Molecular Probes® Streptavidin-conjugated Alexa 594 (Invitrogen Life Science). After thorough washing with PBS-T the sections were mounted with Vectashield Mounting Medium H-1000 (Vector Laboratories).

2.2. Expression and purification of recombinant SCCA1

The construct pMCSG7 serpin B3 (Clone ID: HsCD00343153) was purchased from "DNASU plasmid repository" at the Biodesign Institute/

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http://dx.doi.org/10.1016/j.bbadis.2014.02.005
0925-4439

Arizona State University. Recombinant SCCA1 (serpin B3) was expressed in *Escherichia coli* BL21 (DE3) RIPL (Stratagene). The bacterial pellet was resuspended in 50 mM Na_2PO_4, 300 mM NaCl, 10 mM imidazole, and 10 mM β-mercaptoethanol pH 8 and lysed by sonication on ice. Expressed proteins were purified using Talon superflow metal affinity resin (Clontech). Fractions containing His-SCCA1 were buffer exchanged using Amicon Ultra centrifugal filters (Millipore) with a molecular cutoff of 10 kDa. The protein was further purified using HiTrapQ (GE Healthcare) and a 20 min gradient from 20 mM Tris–HCl pH 8, 20 mM NaCl to 20 mM Tris–HCl pH 8, and 0.5 M NaCl. Fractions containing pure SCCA1 were pooled, snap-frozen in liquid nitrogen and stored at −80 °C.

2.3. Preparation of scale extract and generation of Pso p27 from recombinant SCCA1

Psoriatic scale (300 mg) was homogenized in 10 ml PBS by using an UltraTurrax T25 (Rose Scientific Ltd.). The crude extract was centrifuged at 16,000 ×*g* for 10 min and 1 ml of the supernatant was applied on CnBr-act Sepharose 4B (Amersham Biosciences) coupled with monoclonal anti-Pso p27 antibodies. The extract was repeatedly passed through the immunosorbent column for elimination of the majority of Pso p27 present in the scale extract. The final effluent was used for enzymatic digestion. Digestion was performed in PBS at 37 °C and the reaction mixture contained effluent corresponding to scale extract diluted 1/800 and 1 μg/ml recombinant SCCA1. Quantification of Pso p27 antigen was performed by sandwich-ELISA using specific mouse monoclonal antibodies against Pso p27 [10].

2.4. Incubation of recombinant SCCA1 with psoriatic scale extract (Fig. 1D)

The following samples were incubated for 3 days at 37 °C:

a) Recombinant SCCA1
b) Pso p27 depleted scale extract diluted 1/800
c) Recombinant SCCA1 and Pso p27 depleted scale extract diluted 1:800.

2.5. Incubation of recombinant SCCA1 with recombinant chymase and tryptase (Fig. 2C)

The following samples were incubated for 3 days at 37 °C:

a) Recombinant SCCA1 and human recombinant tryptase (MyBioSource), w/w ratio 100:1
b) Recombinant SCCA1 and human recombinant chymase (Sigma-Aldrich) w/w ratio 100:1
c) Recombinant SCCA1.

2.6. Gel-electrophoretic analyses

The samples were analysed with SDS-polyacrylamide gel electrophoresis (SDS-PAGE) by running 4–12% NuPage Novex Bis–Tris

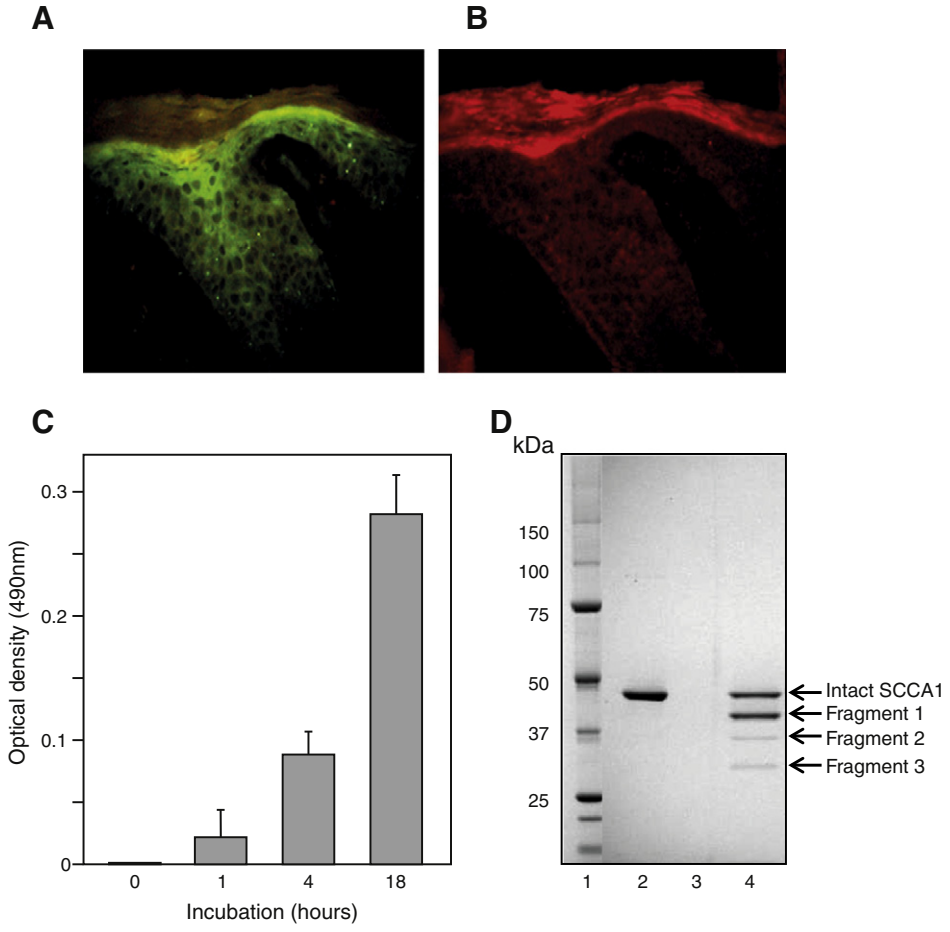

Fig. 1. (A and B) SCCA1 and Pso p27 are spatially separated in psoriatic skin. Double labelling of a thin section of a psoriatic plaque detecting SCCA1 antigen (A) and Pso p27 antigen (B). (C) ELISA quantification of Pso p27 antigen formed by incubation of SCCA1 with psoriatic scale extract. Recombinant SCCA1 and scale extract was incubated at 37 °C for 0, 1, 4 and 18 h, respectively. (D) SDS-PAGE demonstrating intermediate proteolytic fragments of SCCA1. Lane 1; protein MW standard, lane 2; recombinant SCCA1, lane 3; Pso p27-depleted psoriatic scale extract, lane 4; SCCA1 incubated with Pso p27-depleted psoriatic scale extract.

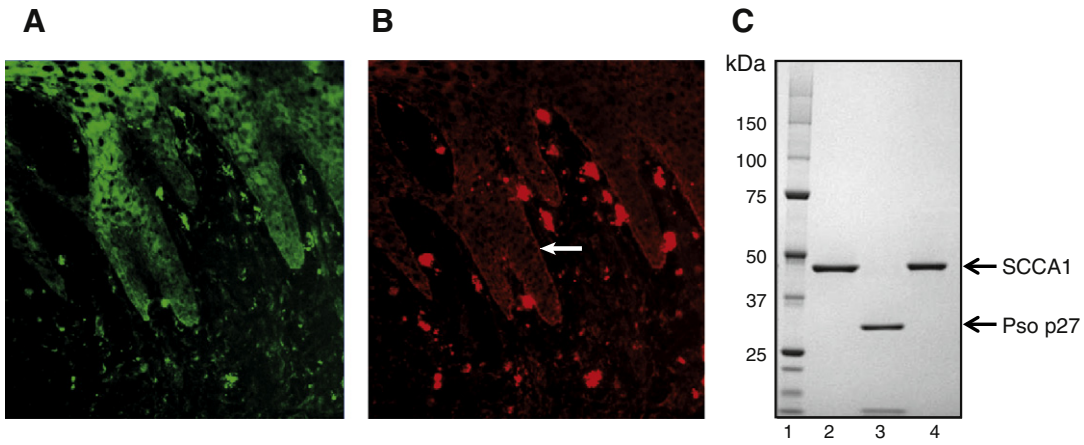

Fig. 2. (A and B) SCCA1 and Pso p27 are present in the same dermal cells in psoriatic skin. Double labelling of a thin section of a psoriatic plaque detecting SCCA1 antigen (A) and Pso p27 antigen (B). (C) SDS-PAGE demonstrating the digestion of recombinant SCCA1 in the presence of chymase. Lane 1; protein MW standard, lane 2; recombinant SCCA1 + tryptase, lane 3; recombinant SCCA1 + chymase and lane 4; recombinant SCCA1.

acrylamide gels in MOPS running buffer (Novex by Life Technologies). The gels were stained with Simply Blue Safe Stain (Novex by Life Technologies).

2.7. MALDI MS/MS analysis of excised protein bands

The protein bands containing about 1 μg protein were excised and in-gel digested [11] with Sequence Grade Modified Trypsin (Promega), Endoproteinase Lys-C (Sigma) or Endoproteinase Glu-C (New England Biolabs). The peptides were further extracted from the gel and desalted using Stage Tip purification [12]. The purified peptides were mixed with an equal volume of 10 mg/ml 2,5-dihydroxybenzoic acid (DHB), air dried on a stainless steel sample stage and analysed on an Ultraflex III TOF/TOF (Bruker Daltonics) mass spectrometer.

3. Results

3.1. Generation of antigen Pso p27 from recombinant SCCA1 with scale extracts

Indirect immunofluorescence of psoriatic skin lesions using a rabbit antiserum against the N-terminal end of the SCCA and a specific monoclonal antibody against Pso p27 is shown in Fig. 1. A strong green fluorescence representing the SCCA molecules was observed in the epidermis with a markedly weaker signal in epidermal cells close to the surface (Fig. 1A). Conversely, a strong red fluorescence representing Pso p27 was observed in the epidermal cells close to the surface and the scale whereas this signal was markedly weaker in the deeper layer (Fig. 1B).

The markedly differential localization of the SCCA and Pso p27 in the epidermal compartments suggested the presence of an endoproteolytic SCCA-targeting factor at the skin surface layer. To investigate this further we set up a quantitative ELISA-based cleavage assay to monitor potential conversion of purified, recombinant SCCA1 to Pso p27 by incubation with extract from psoriatic scale.

The purified SCCA1 protein was not recognized by the monoclonal anti-Pso p27 antibodies (Fig. 1C, time 0). When SCCA1 was incubated with Pso p27-depleted psoriatic scale extract the sandwich ELISA demonstrated significant generation of Pso p27 antigen and that the amount of Pso p27 increased with prolonged incubation times (Fig. 1C).

To further study if the generation of Pso p27 was a result of enzymatic digestion of SCCA1 with specific endoproteases, the digested SCCA1 was analysed by SDS-PAGE. As demonstrated in Fig. 1D, no cleavage products of SCCA1 were observed in the purified SCCA1 substrate (Fig. 1D, lane 2). Moreover, neither intact SCCA1 nor cleavage products were observed in Pso p27-depleted scale extract (Fig. 1D, lane 3). 3 novel forms of SCCA1 with increased electrophoretic migration were observed after 18 h of incubation with scale extract, with a concomitant reduction of intact SCCA1 (Fig. 1D, lane 4). MALDI MS/MS mass spectrometry analysis of the 3 novel forms identified them to be C-terminally truncated SCCA1 (fragment 1), N-terminally truncated SCCA1 (fragment 2) and both C- and N-terminally truncated forms of SCCA1 (fragment 3), corresponding to the Pso p27 protein.

3.2. Generation of Pso p27 from recombinant SCCA1 with chymase

The concomitant presence of SCCA1 and Pso p27 in the same cells indicated an intracellular generation of Pso p27 (Fig. 2A and B). As these cells have been shown to be mast cells [7], the mast cell associated endoproteases, tryptase and chymase, were potential candidates for the transformation. SDS-PAGE analysis of recombinant SCCA1 after incubation with tryptase and chymase, respectively, is demonstrated in Fig. 2C. The SCCA1 molecules were digested in the presence of chymase to a fragment with the same apparent molecular mass as fragment 3 in Fig. 1D.

MS analyses of this new fragment showed N-terminal and C-terminal ends in accordance with those described for Pso p27 [6] and fragment 3 obtained when recombinant SCCA1 was incubated with scale extract (Fig. 1D).

3.3. Conformational consequences when Pso p27 is generated from SCCA1

The N- and C-terminal cleavage points of the SCCA at Tyr^{73}/His^{74} and Val^{349}/Val^{350} (Fig. 3A) both reside in regions of the SCCA crystal structure that have weak electron density [13] and that likely constitute flexible loop regions at the protein surface. The N-terminal fragment of 73 residues contain three α-helices of which α1 and α2 reside at the junction between beta-sheet A (βA) and beta-sheet B (βB) and render (βB) solvent exposed (Fig. 3B). Additional removal of the C-terminal 41 residues would eliminate two strands of beta-sheet B as well as most of the reactive centre loop (RCL) that is essential for cysteine protease inhibition [14] as well as for inhibition of the JNK1 kinase activity [13]. A hypothetical 3-dimensional model of Pso p27 based on the SCCA1 crystal structure (PDB ID: 2ZV6) is presented in Fig. 3B. However, it is very likely that removal of the N- and C-terminal fragments of SCCA1 is accompanied by pronounced structural destabilization of the core (Pso p27) region of the protein. Further work to investigate the structural implications associated with proteolytic cleavage of SCCA1 is now in progress in our laboratory.

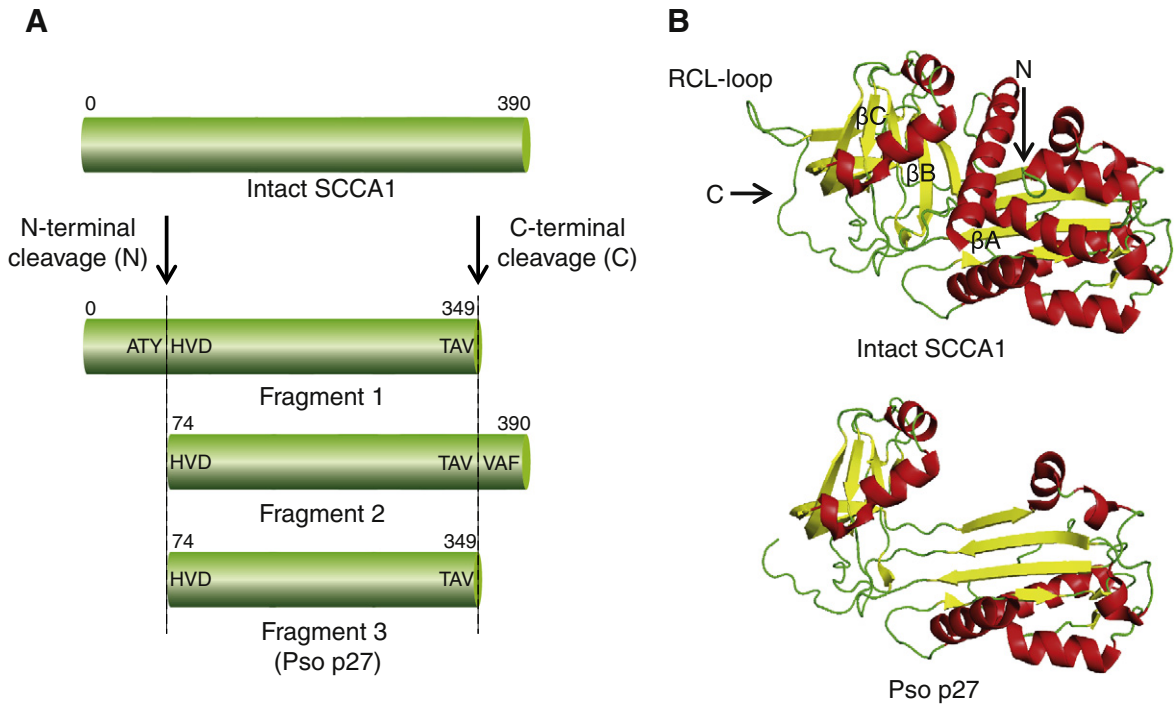

Fig. 3. (A). Schematic illustration of the cleavage products of SCCA1as determined by MALDI MS/MS analyses. (B) Crystal structure of intact SCCA1 [13] (PDB ID: 2ZV6). Arrows indicate the C-terminal and N-terminal (within the RSL) cleavage points (upper panel) as well as the three serpin-typic β-sheets and the reactive centre loop (RCL). The lower panel models SCCA1 74-349 lacking both the N- and C-terminal fragments and corresponding to fragment 3 (Pso p27) (based on PDB ID: 2ZV6).

4. Discussion

Much effort has been put forward in the search for etiological agents associated with chronic inflammatory — or autoimmune diseases. Psoriasis is a chronic inflammatory skin disease which shares many of the features of other autoimmune diseases. The typical psoriatic plaques have well defined boundaries with the surrounding skin. The chronicity of these inflamed lesions argues for a local generation of an auto-antigen. During the last decades we have focused on a protein, Pso p27, associated with psoriasis [4,5,8]. Pso p27 is expressed in psoriatic lesions and is not detected in uninvolved psoriatic skin or skin biopsies from healthy controls [5,8,9]. Through analysis of antibodies obtained from psoriatic scale, we have demonstrated the potential role of Pso p27 as an auto-antigen in psoriasis [3,4].

Based on sequence homologies between Pso p27 and the SCCA proteins we have hypothesized that Pso p27 is generated by post-translational modifications of the SCCA molecules [6]. The concurrent presence of the SCCA and Pso p27 in dermal cells in psoriatic lesions substantiates the assumption of a posttranslational generation of Pso p27 from the SCCA [7].

In this communication we demonstrate that Pso p27 is generated from recombinant SCCA1 when incubated with extracts from psoriatic scale. Interestingly, the proteolytic processing generates two distinct intermediate fragments, omitting either the N-terminal end or the C-terminal end. This strongly suggests that the two digestions are not sequential (Fig. 1D).

Overexpression of SCCA1 in epidermal cells in psoriatic lesions has been described earlier [15] but the expression of the SCCA molecules in Pso p27 positive dermal cells is probably of greater significance with respect to psoriasis pathogenesis [7]. The concomitant presence of SCCA1 and Pso p27 in the same cells indicates an intracellular generation of Pso p27 (Fig. 2A and B). As these cells are shown to be mast cells [5], the mast cell associated endoproteases, tryptase and chymase, are potential candidates for the transformation. The SCCA1 molecules digested in the presence of chymase gave a fragment with the same apparent molecular mass as fragment 3 in Fig. 1D, and MS analyses of this new fragment showed that N-terminal and C-terminal ends are in accordance with those described for Pso p27 [6]. The suggestion of mast cells as antigen presenting cells [16] and the well documented association between psoriasis and selected MHC-I molecules together make the observations described particularly challenging.

The immunological reaction with the Pso p27 specific monoclonal antibodies implies that the N- and/or C-terminal regions in the native SCCA may mediate steric obstruction of the antibody-binding epitopes as indicated in the crystallographic structure and model presented in Fig. 3B. Alternatively, removal of these termini may alter the conformation of the core region of SCCA1 to be recognized by the antibodies.

It has previously been shown that incubation of SCCA1 with papain-like cysteine protease results in cleavage in the RCL loop between Gly^{353} and Ser^{354}, which is four residues away from the cleavage site induced by psoriatic scale extract. Although cleavage within the RCL would imply partial disruption of two of the three β-sheets in the SCCA1 structure, this cleavage did not result in any apparent conformational change of the major cleavage product as determined from circular dichroism analysis [13]. The N-terminal cleavage between Tyr^{73} and His^{74} reported here results in loss of the α1–3 helices that are shielding the third β-sheets from solvent exposure. It is likely that removal of both the N- and C-terminal regions would result in significant alteration in the overall structure of the resulting fragment 3 (Pso p27), but the answer to this must await structural analysis of the Pso p27 protein.

Nevertheless, if we assume that the proteolytic modification of the SCCA molecules is a premise for the antigenicity of Pso p27, a potential therapeutic strategy would be inhibition of the proteolysis. Pso p27 antigen has been shown to participate in the generation of complement activating immune complexes, both in the psoriatic plaques and in synovial fluid from patients with psoriasis arthritis [3,17]. Moreover, Pso p27 antigen has been detected in patients with various inflammatory diseases as for example ankylosing spondylitis [17], sarcoidosis [10] and chronic inflammatory bowel diseases [18]. The proteolytic generation of Pso p27 described here may thus pave the way for new strategies

with respect to search for therapeutic agents against psoriasis and other autoimmune diseases where Pso p27 may play a crucial role [10,17,18].

5. Conclusion

The auto-antigen Pso p27 is generated from SCCA molecules with specific endoproteolytic activity in psoriatic lesions. These findings open up for new therapeutic strategies in psoriasis and probably also in other autoimmune diseases as Pso p27 epitopes have been detected in diseased tissues from patients with various chronic inflammatory diseases.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgements

The work has been supported by grants from the Research Council of Norway. We would like to thank the Proteomics and Metabolomics Core Facility, PROMEC, at NTNU supported in part by the Faculty of Medicine and the Central Norway Regional Health Authority.

References

[1] A.M. Bowcock, J.G. Krueger, Getting under the skin: the immunogenetics of psoriasis, Nat. Rev. Immunol. 5 (2005) 699–711.
[2] M.A. Lowes, A.M. Bowcock, J.G. Krueger, Pathogenesis and therapy of psoriasis, Nature 445 (2007) 866–873.
[3] K. Asbakk, K. Bergh, O.J. Iversen, The psoriasis-associated antigen, Pso p27, participates in the formation of complement activating immune-complexes in psoriatic scale, APMIS 98 (1990) 143–149.
[4] O.J. Iversen, K. Bergh, H. Lysvand, Use of scale antibodies for the detection of antigens in psoriatic lesions, Acta Derm. Venereol. 73 (1993) 31–34.
[5] O.J. Iversen, H. Lysvand, T. Jacobsen, K. Bergh, B.A. Lie, The psoriasis-associated antigen, Pso p27, is expressed by tryptase-positive cells in psoriatic lesions, Arch. Dermatol. Res. 287 (1995) 503–505.
[6] O.J. Iversen, H. Lysvand, L. Hagen, The autoantigen Pso p27: a post-translational modification of SCCA molecules, Autoimmunity 44 (2011) 229–234.
[7] O.J. Iversen, H. Lysvand, The Autoantigen Pso p27 is Generated from SCCA Molecules in Psoriatic Plaques, WebmedCentral DISEASE MECHANISMS, 2012.
[8] M. Dalaker, T. Jacobsen, H. Lysvand, O.J. Iversen, Expression of the psoriasis-associated antigen, Pso p27, is inhibited by cyclosporin A, Acta Derm. Venereol. 79 (1999) 281–284.
[9] P. Song, H. Lysvand, Y. Yuhe, W. Liu, O.J. Iversen, Expression of the psoriasis-associated antigen, Pso p27, is inhibited by traditional Chinese medicine, J. Ethnopharmacol. 127 (2010) 171–174.
[10] T. Jacobsen, B.A. Lie, H. Lysvand, M. Wiig, H.B. Pettersen, O.J. Iversen, Detection of psoriasis-associated antigen Pso p27 in sarcoidosis bronchoalveolar lavage fluid using monoclonal antibodies, Clin. Immunol. Immunopathol. 81 (1996) 82–87.
[11] A. Shevchenko, M. Wilm, O. Vorm, M. Mann, Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels, Anal. Chem. 68 (1996) 850–858.
[12] J. Rappsilber, M. Mann, Y. Ishihama, Protocol for micro-purification, enrichment, pre-fractionation and storage of peptides for proteomics using StageTips, Nat. Protoc. 2 (2007) 1896–1906.
[13] B. Zheng, Y. Matoba, T. Kumagai, C. Katagiri, T. Hibino, M. Sugiyama, Crystal structure of SCCA1 and insight about the interaction with JNK1, Biochem. Biophys. Res. Commun. 380 (2009) 143–147.
[14] C. Schick, D. Bromme, A.J. Bartuski, Y. Uemura, N.M. Schechter, G.A. Silverman, The reactive site loop of the serpin SCCA1 is essential for cysteine proteinase inhibition, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 13465–13470.
[15] A. Takeda, D. Higuchi, T. Takahashi, M. Ogo, P. Baciu, P.F. Goetinck, et al., Overexpression of serpin squamous cell carcinoma antigens in psoriatic skin, J. Invest. Dermatol. 118 (2002) 147–154.
[16] N. Gaudenzio, N. Espagnolle, L.T. Mars, R. Liblau, S. Valitutti, E. Espinosa, Cell–cell cooperation at the T helper cell/mast cell immunological synapse, Blood 114 (2009) 4979–4988.
[17] E. Rodahl, K. Asbakk, O.J. Iversen, Participation of antigens related to the psoriasis associated antigen, Pso p27, in immune complex formation in patients with ankylosing spondylitis, Ann. Rheum. Dis. 47 (1988) 628–633.
[18] O.J. Iversen, T. Jacobsen, Chronic inflammatory diseases, Sarcoidosis Vasc. Diffuse Lung Dis. 13 (1996) 66–69.